LEAFHOPPER TRANSMISSION OF WESTERN ASTER YELLOWS AGENT TO POTATO AND CARROT IN EASTERN WASHINGTON

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PREFACE

This is the last of three manuscripts that have been prepared from a study made on the aster leafhopper and the western aster yellows agent in eastern Washington from 1962 to 1965. The rough draft of this manuscript was prepared December 6, 1968, after the first two manuscripts had been accepted for publication. During the interim, this manuscript was reviewed by pathologists and entomologists and completely rewritten four times; little work was done on the manuscript during the summer months when the senior author was busily engaged in fieldwork on other projects.

The experiments were conducted in eastern Washington. However, the results should be equally applicable in similar areas or regions where the western aster yellows disease is a problem. The authors are not aware of any similar research done concurrently or since that reported herein.

ACKNOWLEDGMENT

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INTRODUCTION

In eastern Washington, western aster yellows (AY) has been observed on potatoes, carrots, clover, several ornamental plants, and weed hosts. In 1962, the aster yellows agent infected 35 to 50 percent of carrot plants and 10 to 15 percent of Russet Burbank potatoes grown for the fall crop. However, in 1963 and 1964, only 10 to 15 percent of carrots and 5 to 8 percent of potatoes were infected.

In the field, AY symptoms are sometimes confused with the symptoms of current season leaf roll on potatoes. Raymer and Milbrath [1] observed and described symptoms of AY on Russet Burbank potatoes in central Oregon in 1952, where 40 to 50 percent of the plants in several fields became infected, compared with only 3 percent in 1946.[2]

The source of infestation, local movements, and host plant preferences of the aster leafhopper, *Macrosteles fascifrons* (Stahl), in eastern Washington were studied. The incidence of AY infection in potato and carrot was observed to be closely correlated to the movement and dispersal of the first and second generations of the aster leafhopper. The movement to potato and carrot, when grain and other crops approach maturity, was determined in a separate study by Hagel, Landis, and Aherns.[3]

The present study of AY was made in eastern Washington from 1962 to 1965 to determine how much AY was actually occuring in Russet Burbank potato and carrot fields, and also whether AY was a factor in causing a tuber discoloration usually associated with leaf roll. Since detailed studies of AY have been conducted throughout the United States and the literature is rather extensive, it does not seem necessary to review all of it here. These studies were conducted coincident with the outbreak of aster yellows. The results of these studies will be applicable during subsequent outbreaks in the future.

MATERIALS AND METHODS

A culture of the aster leafhopper was established in the laboratory from leafhoppers collected in the field during September and October. They were maintained throughout the winter. Small wheat, barley, oats, rye, sweet corn, clover, aster, and horseweed plants were grown in a greenhouse and used to rear the leafhoppers. The AY agent used in these studies was obtained from naturally infected horseweed, *Conyza canadensis* (L.) Cronquist, carrot, ladino clover, *Trifolium repens* L., plants removed from the field, from aster leafhoppers collected from clover and potato fields, and also from leafhoppers collected from various weed hosts.[4] The AY agent was tentatively identified by symptoms expressed in aster and horseweed plants. It was then verified when similar symptoms developed in small aster and horseweed plants after infected leafhoppers had fed upon them for a period of time. The identity of the disease was further identified by grafting diseased potato stems to healthy tomato and flowering tobacco plants and comparing the symptoms described in the literature. Cultures of the leafhopper and the AY agent were easily maintained in the laboratory.

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[1] Raymer, W.B., and Milbrath, J.A. The identity and host relations of the potato late-breaking virus. Phytopathology 50: 312-19. 1960.

[2] Milbrath, J.A., and English, W.H. A late-breaking virus disease of potatoes. Phytopathology 39: 463-9. 1949.

[3] Hagel, G.T., Landix, B.J., and Ahrens, M.C. Sources of infestation, local movement, and host preferences of the aster leafhoppers in eastern Washington. Jour. Econ. Ent. (In press.)

[4] See footnote 3.

Test plants for the transmission studies were grown from seed in greenhouses, and cages covered with Saran were used to confine the leafhoppers on the plants. For each transmission study (fig. 1), leafhoppers were transferred from a stock colony to an AY source plant for a feeding period of 7 to 10 days and then to other young horseweed, wheat, barley, or aster plants for at least a 10-day incubation period to insure that the leafhoppers could infect the host plants being studied.

The mortality of the leafhoppers used in transmission studies on potatoes was high, and not more than 4 out of 25 leafhoppers introduced in cages on potato remained alive after 14 days.

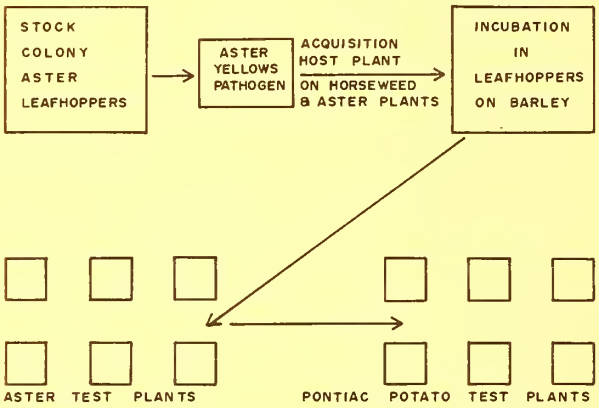

FIGURE 1. — Sequence of AY transmission.

Both approach and scion grants were used in attempts to recover AY. The grafts were bound with soft graft tape and graft compound was used to hasten healing or union of the graft scions and insure the transfer of AY from an infected to a healthy plant. If the transfer of AY were accomplished by grafting, then noninfected leafhoppers were caged on the indicator plant for a time and then transferred to healthy aster plants. If the healthy aster plant developed symptoms of the disease, it was evident that the AY agent was the cause of the disease.

In preliminary tests, we successfully transmitted AY from naturally infected horseweed plants to healthy horseweed plants, red, ladino, and alsike clover plants, and asters, by means of the aster leafhopper. We also transmitted this strain of AY from infected horseweed to Pontiac potato plants with dodder, and by approach grafting we transferred AY to tomato plants. These tests were primarily conducted to develop techniques for producing supplies of test plants and infective leafhoppers according to a planned schedule. In these preliminary trials, only 10 percent of the inoculations were successful.

SYMPTOMOLOGY

The symptoms of AY varied somewhat in different varieties of potatoes. Symptoms were expressed in Pontiac and Kennebec varieties from 21 to 30 days after infection and in Russet Burbank potatoes (fig. 2) from 30 to 42 days. In the Pontiac potato, the foliage became chlorotic followed by vein clearing in the small upper leaves. The infection was often sectional in that some stalks expressed symptoms while others did not, as was observed by Milbrath and English.[5] Usually, the stipules, or bracts in the axils of the branch, became reduced until either aerial tubers or long spikelike adventitious stolons developed, particularly in the Pontiac variety (fig. 3). The foliage of the Kennebec variety became more chlorotic than either the Russet Burbank or the Pontiac. Apical growth stopped shortly after symptoms became evident, and all plants remained alive for 130 days after blossoming, whereas uninoculated plants blossomed at the usual time and the plants continued normal growth. The infected Kennebec plants also developed longer lateral stems than did normal plants.

First symptoms of infection in the Russet Burbank potato were an upward rolling of the apical leaves, retarded terminal growth, and the development of reddish purple pigmentation along the margin of the upper leaves. The plants became stiff and erect with olive green to chlorotic foliage, as observed by Milbrath and English.[6] In the more advanced stages, some plants wilted and died while others continued to grow profusely with dark green foliage. Aerial tubers developed in the leaf axils of some plants.

The first symptom of AY on carrots was a yellowing of young leaves in the crown area. Older leaves became twisted and bronze to reddish in color. Yellowish leaves in the crowns of plants developed a witches-broom appearance which is characteristic of AY. The lateral roots of the plant produced an excess of malformed fibrous shoots and the plants were somewhat stunted.

Infected clover plants showed chlorosis, rosetting and stunting, and phyllody and floral proliferation. The older leaves were usually reddish purple. The affected leaves finally became shriveled and brittle and the entire plant became dwarfed.

Infected horseweed plants have narrow leaves and the innermost leaves of the most

[5]See footnote 2.

[6]See footnote 2.

FIGURE 2. — Aerial tuber symptoms of AY in Russet Burbank potatoes.

rosetted plants are chlorotic and yellow. The outer leaves frequently become bronzed or reddish brown but remain normal in size. Small plants that became infected in the fall and overwintered became completely chlorotic and rosetted with a dwarfed effect. The plants remained alive but grew very slowly and had a bunchy top appearance. Many secondary shoots were produced which added to the rosetted appearance.

FIGURE 3. — Spurlike (unusual) and aerial tuber (usual) symptoms of AY in Pontiac Potatoes. Spurlike symptom on upper left, aerial tuber on upper right.

RESULTS AND DISCUSSION

Leafhopper Transmission Studies

A test was conducted to determine the effect of the number of infective leafhoppers per plant to obtain the percentage of AY agent transfered by the vector to the Pontiac plants, and what percentage were infective.

Tests were made of the ability of lots of 1, 2, 3, 5, 10, 20, and 30 leafhoppers to infect or transmit AY to Pontiac potatoes in the greenhouse. These leafhoppers were first placed on aster or horseweed plants after feeding for at least 10 days on an AY source and then allowing another 10 to 14 days for incubation of the AY agent. Before transfer of the supposedly infected leafhoppers to the potato plants (half-grown), the pretest aster or horseweed plants were examined for AY symptoms. If none had developed, the leafhoppers on that plant were considered to be noninfective and were discarded. Thus, only leafhoppers that were known to be infected with AY were placed on the potato plants. Using this method, the incidence of AY infections on the potato plants could be more closely correlated to the number of leafhoppers used on each plant. The infected potato plants were kept in an insect-free enclosure (greenhouse) to study the rate of AY symptom development and the physiological effects of the disease upon the plants. Parts of the infected Pontiac potato plants were grafted on tomato and *Nicotiana rustica* L. in attempts to recover the disease from the potato plants.

Data presented in table 1 show that when the number of infective leafhoppers was increased from one to three per plant, the percentage of plants infected increased very little. When five insects were used per plant,

Table 1. — Effects of number of AY-infected leafhoppers per plant on the incidence of the disease in lots of 30 Pontiac potato plants in the greenhouse study in 1962

Leafhoppers per plant	*Plants diseased*	*Percent diseased*
1	4	13.3
2	5	16.6
3	8	25.6
5	16	53.3
10	22	73.3
20	25	83.3
30	26	86.6

infection increased considerably. Ten insects per plant resulted in a much higher percentage of infection than with five, but 20 to 30 insects per plant, although increasing infection slightly, were little more effective than 10. Therefore, in all subsequent tests involving transmission, we used 25 infective leafhoppers per plant.

From these results, it seems evident that in a field situation the percentage of infected leafhoppers in a population becomes more of a factor than the number of leafhoppers present. Similarly, host plants in their function as an AY reservoir may become more important than the number of insects in determining the spread of the disease. Some attempts were made during the 3-year period of the study to index groups of leafhoppers as to their effectiveness in AY spread. The leafhoppers were usually found not to be infected with AY until late June or early July, and then only about 1 percent were infective, depending somewhat upon the host plants from which the insects were collected.

Susceptibility of Potato Varieties to AY

Most of these tests were conducted in the greenhouse, but some were done in small field plots.

In the greenhouse, the potatoes were either planted in large pots or directly into the soil in the greenhouse benches. When potatoes were planted in pots, the small plants were caged with Saran-covered cages 8 inches in diameter by 18 inches high, to confine the infective leafhoppers. Each cage had an opening for introducing leafhoppers with a cork stopper to prevent their escape. For the field-plot inoculations, an 18- by 18- by 18-inch cage covered with muslin was placed over each potato plant to confine the leafhoppers. Each cage was fitted with a cloth sleeve for introducing the leafhoppers. These cages were left over the plants for 14 to 21 days to insure that AY infection had occurred. After the cages were removed from the plants in field plots, they were sprayed twice weekly with an effective insecticide until the plants were mature. In the greenhouse, the surviving leafhoppers were destroyed on the test plants, and the greenhouse was kept free of all insects until the plants matured.

Data in table 2 show that during a 3-year period the Kennebec variety was most susceptible to AY followed by Pontiac and Russet Burbank.

Table 2. — Susceptibility of plants of 3 varieties of potato to infection with AY during cage tests with infected leafhoppers, 1962-65

Variety	*Number*	*Percent*
Pontiac	42 per 100	42
Kennebec	12 per 20	60
Russet Burbank	45 per 153	29

Effect of AY on Potato Tubers

In 1962, the effects of AY were determined on Pontiac, Russet Burbank, and Kennebec potato plants grown from certified seed planted in 6-inch pots in the greenhouse. When the plants emerged, 35 leafhoppers from infected horseweed were caged on each plant. Plants that developed AY symptoms were allowed to grow for 120 days, and then the tubers were harvested and examined for internal discoloration. Then slices were taken across the stem end of each tuber and examined after which the tubers were placed in 42° F. storage for 90 to 100 days. The potatoes were then removed from storage and held at room temperature until sprouts developed. Notes were taken of the length and width of the sprouts produced.

In 1963, an experiment was conducted with a series of four Saran-covered cages, 12 by 20 by 8 feet, to try to separate AY and leaf roll symptoms in potato tubers. First, the soil in each cage was fumigated with Telone. Three weeks later, 35 seed pieces from certified seed grade Russet Burbank potatoes were dusted with 5 percent Captan dust and planted in each cage. When all plants had emerged, screen-covered cages, approximately 18 by 18 by 18 inches, were placed over each potato plant in two of the Saran cages, and 25 infective leafhoppers were placed in each cage. The small cages were required to keep the leafhoppers confined near the plants. In another Saran cage, approximately 175 green peach aphids, *Myzus persicae* (Sulzer), were removed from a leaf roll infected potato plant and distributed on the small potato plants, and in one of the two Saran cages stocked with infected leafhoppers a similar number of leaf roll infected aphids were added to the plants. No aphids or leafhoppers were added to the fourth or control cage. After about 120 days, the potato tubers were harvested from all four cages, examined for internal discoloration, then stored for 90 days at 42° F. and removed and kept at room temperature until sprouts appeared.

From the Saran screen field cage tests and greenhouse studies, it was concluded that AY definitely discolored tubers of Pontiac and Russet Burbank varieties but not the Kennebec variety (table 3).

Although List[7] described the internal discoloration in potato tubers infected with AY as resembling that of leaf roll, the discoloration in our study consisted of many light-brown strands radiating in and out from the vascular ring area, often with a number of coarse heavy strands intermixed with the narrower ones. The

[7]List, G.N. Some relationships of insects to net necrosis of the potato in Colorado. Jour. Econ. Ent. 40: 107-12. 1947.

Table 3. — Susceptibility to tuber discoloration of 3 varieties of potatoes when infected with AY in the greenhouse and in field cages and to AY plus leaf roll in field cages

Potato variety, treatment, and year	Percentage of harvested potatoes showing internal discoloration			
	None	Mild	Moderate	Severe
	Greenhouse tests in 1962			
Pontiac:				
Infected with AY in cages	41	34	19	6
Uninfected	96	4	0	0
Russet Burbank:				
Infected with AY in cages	66	17	15	2
Uninfected	79	21	0	0
Kennebec:				
Infected with AY in cages	100	0	0	0
Uninfected	100	0	0	0
	Saran cage field tests in 1963			
Russet Burbank:				
Infected with AY	70	27	3	0
Infected with leaf roll	25	30	22	23
Infected with AY and leaf roll	29	27	23	21
Uninfected	76	22	2	0

internal discoloration was more severe in infected Russet Burbank than in Pontiac potatoes (fig. 4). In many of the tubers, the skin at the stem end was soft and wrinkled (flabby). Although a small amount of discoloration usually occurs as a narrow circle in the vicinity of the vascular ring in most Russet Burbank tubers from some unknown cause, there were 6 percent more tubers with internal discoloration in the Russet Burbank potatoes infected with AY in the Saran cage than in the check cage. When both leaf roll and AY were present in Russet Burbank potatoes, the tuber discoloration was similar to that of leaf roll alone.

FIGURE 4. — Necrosis type of discoloration caused by AY in Russet Burbank potatoes.

Seasonal Incidence by AY in Carrot and Potato Fields

In 1961, and again in 1962, a number of horseweed plants that had germinated in late summer and fall in fields in the Columbia Basin were transplanted to 6-inch metal pots and held in an insect-free greenhouse during the winter to determine the amount of AY infection that had occurred in the field before transplanting.

Horseweed plants that had become infected with AY in the field during the fall showed no symptoms until spring, and then the symptoms of the disease persisted throughout the following summer. However, when transplanted from the field to the greenhouse in the fall, AY symptoms developed rather quickly. Based on results from small horseweed plants that were removed from the field in the fall and grown in the greenhouse during winter, the field infection with AY in the fall of 1962 was 72 percent and in 1963, 34 percent. Infected plants that remained over winter in the field developed AY symptoms in June and July, and as the plants matured, the symptoms became more pronounced.

In 1962, 1963, and 1964, several fields of carrots and Russet Burbank potatoes in the Columbia Basin were examined at intervals to determine the incidence and rate of symptom expression during the summer. One hundred plants were examined in 3 acres. They consisted of 10 carrot and 10 potato fields each and were examined twice weekly from June 1 to September 1. In 1963 and 1964, 30 healthy aster plants were placed in each of three potato fields during the period from June 1 to August 31. The plants

were grown in the greenhouse, transplanted in the potato fields, and left for 2 weeks. Then 30 new plants were taken to the field and the old ones brought back to the greenhouse where they were kept free of insects and held for AY symptom development. The symptoms were recorded as they developed.

The incidence of AY in carrots and potatoes in the field was determined by comparing symptoms of diseased plants grown in the laboratory with symptoms found in plants in the field and also by transplanting aster plants in the field at 2-week intervals. Infected carrot plants were also taken from the field. They were either transplanted in pots in the greenhouse where uninfected leafhoppers were caged on them for 7 days and then transferred to healthy aster plants, or stems of field-infected potato plants were grafted to healthy tomato plants or to *Nicotiana rustica* L. from which non-infective leafhoppers acquired the AY agent and then transferred it to either aster or horseweed plants. Data on the time of infection of AY by leafhoppers on transplanted aster plants in the field indicated that infection began in late June and continued through July and August (table 4).

Table 4. — The seasonal incidence of AY infection found in weekly counts in commercial crops of carrots and potatoes and biweekly counts on aster plants after having been transplanted in potato fields in eastern Washington in 1962-64

Year, crop or plants	Percentage of plants showing AY symptoms during indicated periods[1]				
	6/6-6/20	6/20-7/5	7/5-7/18	7/18-8/1	8/1-8/15
1962:					
Potato	0	0	0	5.0	15.0
Carrot	0	0	10.0	35.0	50.0
1963:					
Aster	0	0	14.8	10.0	6.2
Carrot	0	0	1.0	3.0	10.0
Potato	0	0	1.0	3.0	5.0
1964:					
Aster	0	6.6	15.0	10.0	0
Carrot	0	0	2.0	4.0	5.0
Potato	0	0	1.0	5.0	8.0

[1] Accurate identification of AY symptoms is difficult on potatoes early in the season. Foliage symptoms were read directly in the field except on asters which were brought back to the laboratory after exposure in the field.

SUMMARY

The aster leafhopper, *Macrosteles fascifrons* (Stäl), transmitted AY pathogen from naturally infected carrot, red, alsike, and ladino clover and horseweed, *C. canadensis* (L.) Cronquist, to aster and horseweed plants grown from seed and to potato in greenhouse trials. The AY pathogen was also transmitted from naturally infected horseweed to Pontiac potato by dodder and by grafting infected potato stems to tomato and *N. rustica* L.

Pontiac potato plants following infection with AY, developed symptoms similar to those described for purple top wilt. Some young Russet Burbank potato plants infected with AY wilted and died prematurely, whereas other infected plants attained near normal growth. Phyllody was evident in some Russet Burbank plants. Field infection with AY in potatoes occured mostly in July and August.

The pathogen was transmitted in laboratory cages and greenhouses by approach grafts. It was also transmitted by stem grafts from naturally infected potatoes to tomato and from 12 of the diseased plants to *N. rustica*, and to three of 10 aster scions.

Typical AY symptoms consisted of the plants becoming stiff and erect with olive-green to chlorotic foliage. Yellowing and cessation of growth of the interveinal tissues of the youngest leaf, while the midrib and lateral vein tissue continued to grow, resulted in a rolling and curling of the leaf. Leaves became dwarfed, with short petioles. The plants showed a bunchy top condition within 6 weeks of inoculation.

This AY pathogen was readily transmitted to aster, horseweed, and to carrot or clover with the aster leafhopper.

Incidence of the AY disease in the field was correlated with environmental factors as well as food supply governing the abundance of the aster leafhopper. Among them were suitable climate and cropping conditions for development and successful overwintering of the egg stage, also the favorable conditions for abundant plant survival with an increased abundance of a reservoir from which leafhoppers could acquire the pathogen in the following season. In Oregon, Raymer[8] and Raymer and Amen[9] associated an outbreak of AY to an increase in commercial production of ladino clover. These circumstances favored the development of the aster leafhopper and plant hosts of AY pathogen within the area.

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8 Raymer, W.B. Identity and host relations of the potato late-breaking virus. (Abs.) Phytopathology 46: 639. 1956.

9 Raymer, W.B., and Amen, C.R. An association of late-breaking virus in potato with a phyllody condition in ladino clover. (Abs.) Phytopathology 44: 503. 1954.

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